Vol. XXVII. *November, 1914.* *No. 5*

BIOLOGICAL BULLETIN

IS THE FERTILIZATION MEMBRANE OF ARBACIA EGGS A PRECIPITATION MEMBRANE?

E. NEWTON HARVEY,

PRINCETON UNIVERSITY.

In 1912 McClendon ('12) suggested that the fertilization membrane of sea urchin eggs is a precipitation membrane formed when two colloids of opposite electrical charge meet, namely the negative egg jelly (mucous, zona pellucida) and a positive substance secreted by the egg. Elder ('13) has advanced a similar view and recently McClendon ('14) has restated his former opinion. Of course the test of the theory is perfectly simple. An unfertilized egg from which the jelly has been removed should form no membrane when fertilized, and both McClendon and Elder state that this is the case.

I have utterly failed to confirm these statements and find that whether an egg forms a normal membrane or not is absolutely independent of the jelly which surrounds it. My method of determining this is as follows:—Eggs from one female are divided into two parts and the jelly removed from the eggs of one part by shaking two or three times in a test-tube and washing with sea water. Eggs of both lots are placed together upon a slide and india ink suspension, sperm, and, after mixing, a cover glass is added. It is perfectly easy to see which eggs are surrounded by jelly and which are not. All of them, whether with or without jelly, form fertilization membranes which in the two-cell stage surround the whole egg and are quite distinct from the hyaline plasma layer which is close to each blastomere. An egg without jelly touching the jelly of another egg should form a membrane, according to McClendon's idea, only on the side toward the jelly. Yet such a condition is never observed, but instead a membrane forms about the whole egg. Thus eggs without jelly will form fertilization membranes.

If *Arbacia* eggs are allowed to stand for about 52 hours at a temperature of 22° C. or for 3 to 4 days at a temperature of 12° C. no membranes form despite the fact that they may still be surrounded by a copious amount of jelly. This observation so far as I am aware was first made by Loeb ('03) and confirmed by myself ('10) and F. R. Lillie ('14). In the two-cell stage the blastomeres are widely separated because not surrounded by a fertilization membrane although the hyaline plasma layer is clearly visible.[1] Thus even an egg surrounded by jelly may fail to form a fertilization membrane.

I at first thought that McClendon's observations were due to the fact that he took so long a time in removing the jelly, (agitation and washing for "not more than 24 hrs.") that the mere age of the eggs would account for their inability to form membranes. I find, however, that the eggs *with jelly* must stand for over 52 hrs. at 22° C. before they are unable to form a fertilization membrane.

This fact suggests that the membrane-forming substance (membranogen) which passes out of the egg to form the membrane gradually diffuses away or is used up when the egg is allowed to stand. Since the membranogen is probably a protein we should expect it to diffuse away from the eggs without jelly much more readily than from those eggs with jelly. It is well known that colloids do not readily diffuse through each other. Such is actually the case and in this point lies, I believe, the explanation of McClendon's results. If we take the eggs from one female, remove the jelly from one lot by shaking, but allow it to remain on the other lot, both lots will form perfectly normal membranes if fertilized immediately. If both lots are allowed to stand for 24 hrs. and are then fertilized the eggs which have stood without jelly form no membranes while those with jelly form membranes only slightly thinner than normal. Membrane

[1] The hyaline plasma layer appears much thicker than in freshly fertilized eggs and in my previous paper ('10) I described this as a special type of membrane. However there is nothing present at all comparable to the normal fertilization membrane and without quibbling over minute distinctions we may safely class these eggs as "without membranes." McClendon ('14) misquotes me in stating that I believe the fertilization membranes to be present on unfertilized eggs and to be simply lifted off after fertilization. I have held exactly the opposite view (Harvey, '10).

formation is not, then, a question of presence or absence of jelly but is dependent on the time the eggs have stood. We may imagine this to be due to the loss of some membrane-forming material from the eggs which much more readily takes place when the mechanical hindrance of the jelly is removed.[1]

These experiments point against the view that the fertilization membrane is a precipitation membrane.

BIBLIOGRAPHY.

Elder, —.
'13 Arch. f. Entwick., 35, 195.
Harvey, E. N.
'10 J. Exp. Zool., 8, 365.
Kite, G. L.
'12 Science, n. s., 36, 562.
Lillie, F. R.
'14 J. Exp. Zool., 16, 550.
Loeb, J.
'03 Pflüger's Arch., 365, 59.
McClendon, J. F.
'12 Science, n.s., 33, 387.
'14 Zeit. f. physiol. chem. biol., 1, 163.

[1] According to Kite ('12) membrane formation is merely the swelling of a fine invisible (unless stained) vitelline membrane together with a change of its optical properties. If that is true the inability of eggs which have stood for some time without jelly to form fertilization membranes would seem to be due to a loss, through solution, of the vitelline membrane.